ALDOLASE IN BOVINE MILK

B. D. POLIS AND H. W. SHMUKLER

Eastern Regional Research Laboratory,[1] *Philadelphia 18, Pennsylvania*

The enzyme aldolase which reversibly splits fructose 1,6-diphosphate into dihydroxyacetone phosphate and phosphoglyceric aldehyde was first discovered by Meyerhof and Lohmann (3) in rabbit voluntary muscle. The enzyme probably occurs in all cells, but muscle and yeast are the best sources. More recently, the enzyme has attracted attention because the experiments of Warburg and Christian (6) indicated an increase of aldolase in the serum of tumor-bearing rats. With the known relationship of the serum and milk whey proteins (2) in mind, it was of interest to determine the possible presence of aldolase in normal milk.

EXPERIMENTAL

The procedure described in detail by Sibley and Lehninger (5) was applied directly to milk without any modifications. The method depends on the formation of a 2,4-dinitrophenylhydrazine derivative of the triose phosphate produced by the action of aldolase on hexosediphosphate. In alkaline solutions, the dinitrophenylhydrazine derivative, called chromogen, turns purple. The assay of whole or skimmilk for aldolase activity was complicated by turbidity in the solution of the chromogen after addition of NaOH. This occurred with normal milk or milk inactivated by trichloroacetic acid. The turbidity did not appear with milk that had been dialyzed or with milk aldolase preparations made by salt fractionation. To eliminate the turbidity, the colored solution was centrifuged immediately before it was compared with acid-inactivated milk treated in a similar manner. The aldolase activity of milk determined by this procedure was identical with that of a dialyzed sample of the same milk. No further difficulty was encountered with the aldolase assay.

Aldolase activity may be conveniently expressed as the micromoles of 1,6-fructose diphosphate split by 1 mg. of protein at 37° C. in 1 hr. One micromole of hexosediphosphate is equivalent to 2 micromoles of triosephosphate or 2 micromoles of alkali-labile phosphate. The equivalence between the triose chromogen and alkali-labile triosephosphate was determined with a rat-muscle preparation of aldolase. The aldolase activity, stated in terms of micromoles of hexosediphosphate split, multiplied by the factor 22.4 is equivalent to the Q_{HDP} used by Sibley and Lehninger (5). For comparison with their data, this Q notation is used.

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. D. A.

Protein concentrations were determined by the biuret color reaction described by Kingsley (1). Milk was analyzed within a few hours after collection or after storage overnight at 3° C. in the presence of chloroform. Both mixed commercial skimmilk from a Philadelphia dairy and whole milk from each of six animals were analyzed. The results are summarized in table 1.

TABLE 1

Aldolase activity of normal milk and various milk fractions

	Q_{HDP}	
	Range	Average
Whole milk[a]	0.07–0.13	0.09
Cream[b]	0.14–0.45	0.34
Skim milk[c]	0.07–0.22	0.13
Whey (casein precipitated with acid)	.....	0.00
Whey (casein coagulated with rennet)	0.15–0.35	0.22
Rennet whey salt fractionation at 26° C.		
2.3 *M* ammonium sulfate ppt.[d]		0.41
2.8 *M* " " "		0.00
Salt fractionation at 3° C.[e]		
2.4 *M* ammonium sulfate ppt.[d]		2.3
2.8 *M* " " "		6.8

[a] Whole milk from each of four cows was analyzed.

[b] Whole milk was centrifuged for 10 min. at room temperature (3000 rpm), and the skimmilk was siphoned off. One ml. from the center of the recentrifuged cream layer was used for the aldolase determination.

[c] From two 15-gal. lots of mixed unpasteurized commercial skimmilk and eight lots of milk from six cows.

[d] The fractions were dialyzed free of salt before enzyme assay. The pH was then 6.4 ± 0.1.

[e] Casein was precipitated at 1.5 *M* $(NH_4)_2SO_4$ concentration.

RESULTS AND DISCUSSION

The activity of milk aldolase is of the same order as that reported for blood serum (5). Like xanthine oxidase, this milk enzyme is concentrated in the cream layer. Use of a conventional salt fractionation procedure at room temperature was complicated by the instability of the enzyme in milk. Removal of casein by isoelectric precipitation at pH 4.7 resulted in a complete acid inactivation of the whey aldolase. Although there was an apparent increase of the Q_{HDP} value when the casein was removed with rennet and the enzyme was precipitated at a concentration of 2.3 molar $(NH_4)_2SO_4$ at room temperature, there was a loss of almost two-thirds of the total enzyme activity. The removal of casein with 1.5 *M* $(NH_4)_2SO_4$ and the subsequent fractionation of the whey at 3° C. concentrated the milk aldolase in the fractions that precipitated at concentrations of 2.4 and 2.8 molar salt. Approximately 80 per cent of the total activity in milk could be recovered with this procedure. In the fraction that precipitated at a concentration of 2.8 molar $(NH_4)_2SO_4$ there was about a fifty-fold increase in purity ($Q_{HDP} = 6.8$).

Some explanation for the loss of activity with the rennet whey was obtained in the subsequent study of the effect of temperature on the stability of the milk aldolase. Figure 1 demonstrates a marked instability of the enzyme in milk at 37° C., as compared with the stability of the relatively purified milk aldolase. The

linear relationship for the plot of the log per cent aldolase activity remaining after heating against the time of heating reveals a simple monomolecular inactivation rate for the enzyme in milk at 37° C. A similar curve was obtained with dialyzed milk, indicating that the instability of the aldolase in milk could not be attributed to dialyzable components. Although the purified aldolase fraction showed no inactivation at 37° C., at 48° C. the activity of this fraction diminished rapidly but still followed a monomolecular reaction rate. The complicated inactivation rate of the aldolase in milk at this temperature probably indicated inactivation due to heat and unknown degradative changes.

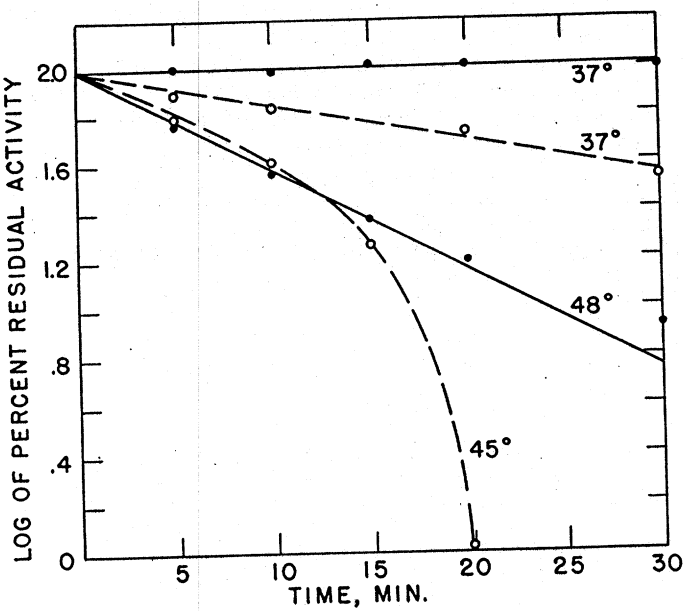

FIG. 1. Aldolase activity of normal milk (O) and a purified milk fraction (●) after heating at various temperatures for progressive time intervals. The pH was 6.4 in both cases. The purified fraction had been dialyzed free of salt.

This rapid inactivation of the aldolase in milk, in the light of the relative stability of the aldolase in fractions from milk, permits the conclusion that milk contains non-dialyzable components capable of destroying or inactivating the aldolase. Similar factors have been found in the crude extracts of muscle aldolase.

The presence of aldolase in milk with an activity level close to that reported for blood serum ($Q_{HDP} = 0.3$) emphasizes further the close relationship of the proteins of serum and milk whey (2). In view of the reported presence of xanthine oxidase in both bovine blood serum and milk and its absence in both human serum and milk (4), the presence of aldolase in both serum and milk constitutes further presumptive evidence of the possible origin of certain milk enzymes.

SUMMARY

With the procedure of Sibley and Lehninger, the enzyme aldolase has been found in normal cow's milk in the same concentration range as in blood serum. The presence of the enzyme in various milk fractions is indicated, and factors affecting the stability of the enzyme in milk are discussed.

REFERENCES

(1) KINGSLEY, G. R. The Direct Biuret Method for the Determination of Serum Proteins as Applied to Photoelectric and Visual Colorimetry. J. Lab. Clin. Med., 27: 840. 1942.

(2) MCMEEKIN, T. L., AND POLIS, B. D. Milk Proteins. Advances in Protein Chemistry, 5: 201–228. Academic Press, N. Y. 1949.

(3) MEYERHOF, O., AND LOHMANN, K. Über die enzymatische Gleichgewichtsreaktion zwischen Hexos diphosphorsäure und Dioxyacetonphosphorsäure. Biochem. Z., 271: 89. 1934.

(4) RODKEY, F. L., AND BALL, E. G. Test for Distinguishing Human from Cow Milk Based on a Difference in their Xanthine Oxidase Content. J. Lab. Clin. Med., 31: 354. 1946.

(5) SIBLEY, J. A., AND LEHNINGER, A. L. Determination of Aldolase in Animal Tissues. J. Biol. Chem., 177: 859. 1949.

(6) WARBURG, O., AND CHRISTIAN, W. Gärungsfermente im Blutserum von Tumor-Ratten. Biochem. Z., 314: 399. 1943.